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14. ABSTRACT

Bone is the frequent metastatic site for human prostate cancer resulting in significant morbidity and mortality in patients with advanced disease. The type of bone defect encountered in prostate cancer bone metastasis is osteoblast lesions resulting in excess bone. However, initiation of osteoclastogenesis is first aided by osteolysis, mediated by osteoclasts. The areas provided as source for osteoblast accumulation later leads to thickening of the bone. In this proposal, we planned to address arresting both the events of osteolysis and osteoblastogeneis by biological inhibitors of these two events. Osteoprotegerin (OPG) is a "decoy" receptor that competes with RANK for RANKL, thus, modulating the effects of RANKL. Thus, OPG remains an effective molecule for future therapies for bone metastasis. We sought to achieve sustained effects of OPG combining cell therapy and gene therapy approaches. Similarly, for inhibiting osteoblast activity we chose noggin, capable of arresting osteoblast formation. The aims were to determine therapeutic effects of OPG and noggin expression by rAAV gene therapy in a murine model of prostate cancer bone metastasis. So far, we produced high-titer recombinant AAV vectors encoding osteoprotegerin, and noggin and currently testing the feasibility of MSC therapy for reducing bone burden initiated by cancer growth. Continuation of the ongoing studies in to next year will provide valuable information on therapeutic effects of this therapy for prostate cancer bone metastasis.

15. SUBJECT TERMS
Osteolysis, osteoprotegerin, noggin, gene therapy, adeno-associated virus

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INTRODUCTION

Bone is the frequent metastatic site for human prostate cancer resulting in significant morbidity and mortality in patients with advanced disease. A vicious cycle, arising due to the interaction of cancer cells and the bone microenvironment results in the upregulation of factors promoting osteoblastogenesis. Presently, it is clear that the event of osteoblastogenesis in prostate cancer bone metastasis is preceeded by osteoclastogenesis. Thus, osteolysis and osteoblastogenesis can be inhibited by interrupting one or more of the steps involved in the cycle.

A better understanding of bone remodeling and molecular events in osteolytic and osteoblastic bone lesions identified the role of key activators and inhibitors of both these events. The receptor activator of nuclear factor κ B ligand (RANKL), produced by osteoblasts, activated T cells and marrow stromal cells stimulates the recruitment, differentiation, and activation of osteoclasts by binding to RANK. Osteoprotegerin (OPG) is a "decoy" receptor that competes with RANK for RANKL thereby modulating the effects of RANKL. Thus, OPG is a promising molecule for inhibiting osteoclastogenesis. On the other hand, noggin, a secreted glycoprotein with proven antagonistic activity on bone morphogenetic proteins (BMP) and osteoblast differentiation will inhibit osteoblastic lesions. To achieve sustained effects of these two molecules, gene therapy is more powerful than pharmacological therapies. Since bone metastasis is a secondary event that occurs in late-stage disease or during recurrence, genetic therapies aimed at controlling this process should be sustained and localized. Thus, for sustained expression of therapeutic levels of OPG or noggin, a vector capable of stable expression of the transgene without vector-associated toxicity and immunity is ideal. The adeno-associated virus vectors (AAV) are more promising to this end. With recombinant AAV, it is possible to obtain significant therapeutic gains by either systemic or bone-targeted transduction using mesenchymal stem cells with bone homing signals.

The central hypothesis of the proposed work is that systemic or bone targeted gene therapy using recombinant AAV and gene-modified mesenchymal stem cell vehicles capable of bone homing and inhibiting osteoclastic and osteoblastic bone lesions in prostate cancer by stable expression of OPG and noggin respectively will be effective treatment modalities for prostate cancer bone metastasis. The proposed studies will seek to identify the effects of OPG and noggin therapy also as a means to delineate the intricate role of osteoclastogenesis and osteoblastogenesis in the progression of prostate cancer bone metastasis. This hypothesis will be evaluated in the present study by using a novel bone-targeted mesenchymal stem cell vehicle, and non-invasive bioluminescent imaging of the implanted prostate cancer cell growth and metastases in SCID mouse.

Specific Aims: 1) To develop and characterize rAAV encoding human OPG and noggin, and clones of PC-3, LAPC-9 and LNCaP cell lines stably expressing luciferase for non-invasive imaging, 2) To determine preventive and therapeutic effects of systemic and bone-targeted OPG expression by rAAV gene therapy in SCID mice with osteolytic, osteoblastic and mixed lesions of prostate cancer bone metastasis, *and* 3) To determine preventive and therapeutic effects of BMP antagonist noggin by rAAV gene therapy in metastatic prostate cancer mice models *in vivo*.

BODY

Production of adeno-associated viral vectors encoding human OPG and analysis of expression as a soluble factor. We constructed recombinant adeno-associated virus vectors (rAAV) encoding osteoprotegrin (OPG), either as a fusion protein to the human immunoglobulin (Fc) or without Fc. The constructs were tested initially for the expression and extracellular secretion of OPG in RAW (a murine macrophage cell line) cell cultures. Results, shown in Figure 1, indicate the expression of OPG from rAAV transduced cells.

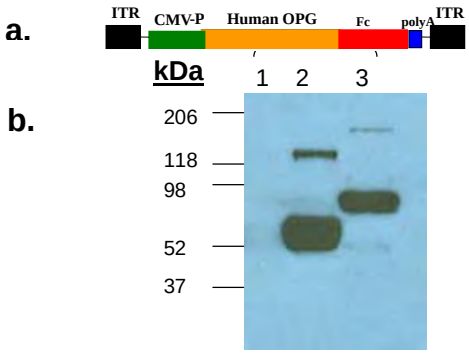

Figure 1. Recombinant AAV encoding the human OPG.Fc (a) and expression of the OPG.Fc from RAW cell supernatant (b). RAW cells were mock-transduced or transduced with rAAV-OPG.Fc construct and the supernatants were analyzed by Western blot using a monoclonal antibody for human OPG. Lane assignments: Lane 1: Mock; 2: s-hOPG-Fc; 3: hOPG-Fc.

Transduction of rAAV-OPG.Fc inhibits osteoclast differentiation *in vitro*. The biological activity of rAAV produced OPG was determined in osteoclast forming assay using RAW cells. Briefly, 10^5 RAW cells were plated in 24-well tissue culture plates and grown in medium containing 10% FBS, 20 ng/ml M-CSF, and 50 ng/ml RANKL in the presence or absence of conditioned medium from 293 cells transduced with rAAV-OPG.Fc. The growth medium plus additives were changed every alternate day. After five days of culture, the cells were fixed and stained for tartrate-resistant alkaline phosphatase (TRAP), a marker for multinucleated osteoclasts. Results, shown in Figure 2, demonstrate that rAAV produced OPG is biologically active and effectively inhibits osteoclastogenesis.

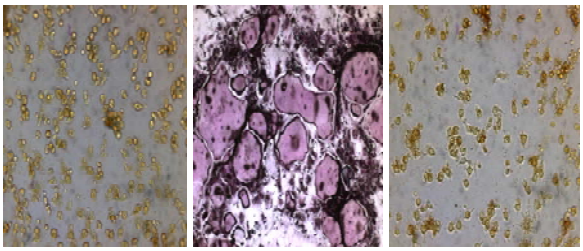

Figure 2. Expression and bioactivity of rAAV-OPG.Fc in vitro. RAW264.7 cells were cultured for 14 days with either 50ng/ml RANKL or combination of RANKL and 125 ng/ml of purified OPG.Fc. A: Control, B: RANKL and C: RANKL +OPG.Fc

In order to achieve bone-specific delivery of gene-modified mesenchymal stem cells producing OPG or noggin, we developed a method to transiently express α1 integrin. Heterodimerization of α1 integrin with endogenous β4 integrin allows the cells to home preferentially to bone as shown in Figure 3.

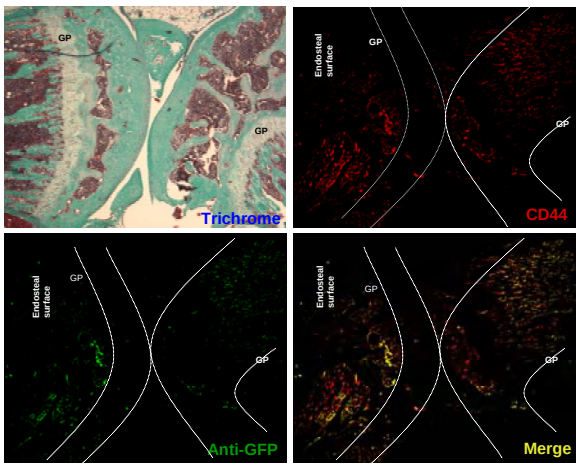

Figure 3. Immunochemistry and immunohistochemistry of mouse limb bones following transplantation with α4 integrin expressing, GFP-positive MSC. Four weeks after transplantation with syngeneic, GFP transgenic mouse MSC, mice were sacrificed and long bones were immunohistochemically stained with anti-GFP and anti-mouse CD44 antibodies. The presence of GFP-positive cells embedded in the primary spongiosa and bone marrow near the growth plate/metaphysis area indicate the regenerating potential of engrafted donor cells. Double-positive cells for GFP and CD44 antibody indicate the presence of undifferentiated MSC. Trichrome staining of the same region indicating the structural organization of the bone was used to mark the endosteal and growth plate (GP) regions after fluorescence microscopy.

To test the efficiency of OPG-expressing MSC in protecting osteolytic lesions due to cancer bone metastasis, encountered commonly in breast cancer patients, we transfected OPG expression vector in mouse MSC and transplanted them to tibial bones of nude mice harboring osteolytic cancer cell line, PC-3. These cells were stably transfected with luciferase gene, hence, allowed non-invasive imaging of the cancer cell growth. Micro-CT analysis of the bone following the therapy indicated remarkable retention of bone architecture after the OPG-expressing MSC were therapeutically implanted. Representative data is shown in Figure 4.

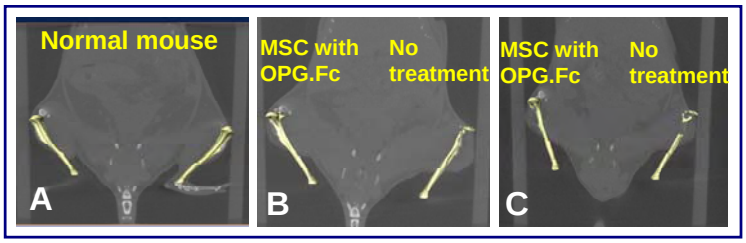

Figure 4. Radiographic images of mice tibia following treatment with MSC producing OPG.Fc. Approximately 10^5 osteolytic bone metastatic prostate cancer cell line PC-3 were implanted in mouse tibia (B & C). Seven days after tumor cell implantation, MSC producing OPG.Fc was injected in one side and the other side left untreated. Picture shown in panel A is from a normal mouse without any tumor or MSC.

KEY RESEARCH ACCOMPLISHMENTS

- Developed rAAV encoding human OPG, produced high-titer virus and validated the biological efficacy of the vector encoded protein in inhibiting osteoclastogenesis in vitro.
- Developed strategy to increase bone-specific homing of MSC.
- Established that MSC expressing OPG greatly reduce ostelytic effects of cancer growth in bone.
- Constructed and produced recombinant AAV encoding noggin for testing its therapeutic effects in inhibiting osteoblastic bone formation in bone metastatic prostate cancer model.

REPORTABLE OUTCOMES
(Papers published or communicated)

Isayeva, I., Chanda, D., Eltoum, I-E., and **Ponnazhagan, S**. Effects of sustained anti-angiogenic therapy in multi-stage prostate cancer in TRAMP mice. Cancer Res. 2007 (in press).

Kumar, S., and **Ponnazhagan, S**. Bone homing of pluripotent mesenchymal stem cells by ectopic α4 integrin expression. FASEB J. 2007 (in revision).

CONCLUSIONS

So far, we produced high-titer recombinant AAV vectors encoding osteoprotegerin and noggin, and tested the feasibility of MSC therapy for reducing osteolysis in bone initiated by prostate cancer growth. We finished producing rAAV encoding noggin for its therapeutic effects on osteoblastic bone complications in a mouse model of prostate cancer. Also we established a method for bone homing of ex vivo cultured MSC by transient expression of α4β1 integrin. Continuation of the ongoing studies in to next year will provide valuable information on therapeutic effects of this therapy for prostate cancer bone metastasis.

PERSONNEL RECEIVING PAY FROM THIS GRANT

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REFERENCES

N/A

APPENDICES

N/A